Johne's Disease

– laboratory testing

A guide for veterinarians and farmers

JOHNE'S DISEASE RESEARCH CONSORTIUM

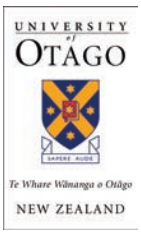
DairyNZ

The Johne's Disease Research Consortium is an Unincorporated Joint Venture established in 2008 with a mandate to reduce the impact of Johne's Disease on farm in New Zealand.

It has as its participants Beef + Lamb NZ Ltd, DairyNZ Inc, DEEResearch Ltd, Massey University, University of Otago, AgResearch Ltd and Livestock Improvement Corporation.

The Meat Industry Association and Dairy Companies Association of New Zealand are associate participants and Landcorp Farming Limited, Johne's Management Ltd and The New Zealand Merino Company Ltd contribute to the research programme.

JDRC receives funding from the Ministry of Business, Innovation and Employment via the Research Partnership scheme.



For more information refer to the DairyNZ guide to Johnes's Disease Management for New Zealand dairy herds by visiting dairynz.co.nz or phone 0800 4 DairyNZ (0800 4 324 7969)

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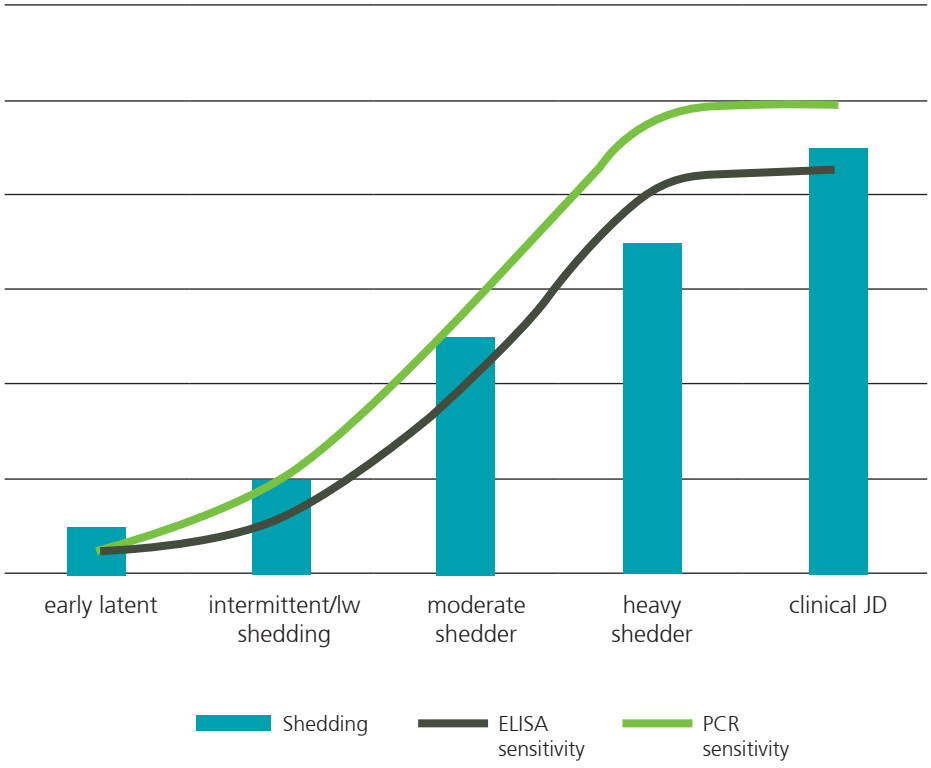
Laboratory testing for Johne's Disease

A dairy herd Johne's management plan must reduce exposure and transmission risks:

- ✓ Develop a whole herd health plan that includes JD testing
- ✓ Screen all cows annually for Johne's before calving
- ✓ Cull all test-positive cows ASAP and calve only test-negative cows
- ✓ If unable to cull, tag positive cows and calve separately. Cull the calves. Discard colostrum.
- ✓ Avoid carry-over cows or test before carrying over (and before calving).
- ✓ Consult test providers for expert advice to interpret results.

The challenges of diagnosing Johne's Disease in a herd

The biology of Johne's infection presents significant challenges for diagnostic tests whether the test is designed to detect the presence of Johne's bacteria (MAP[1]) directly, or detect the host's immune response to the infection. The protracted nature of infection means that the bacteria can remain hidden in the gut wall for many years with little or only intermittent shedding. In the early stages even highly sensitive molecular tests such as the PCR[2] are unable to detect Johne's bacteria in routine clinical samples. Similarly, the humoral immune response measured by serological tests like ELISA[3] develops after local cellular responses have failed to contain the infection and the disease has already progressed. Therefore, testing aimed at identifying the early subclinical stages of Johne's infection is expensive and ineffective in practice. This makes attempts to eradicate Johne's disease (JD) frustrating and unrewarding.



Successful detection of Johne's Disease in a herd

As the disease progresses, Johne's bacteria are increasingly shed in faeces and diagnosis of JD becomes easier and more reliable. Faecal PCR testing enables identification of cattle that are shedding Johne's bacteria and provides a means of assessing disease severity by measuring the amount of bacteria in the sample. At the same time the performance of ELISA tests improves as progression of the disease causes antibody levels to rise. Therefore, careful application of modern tests can identify and rank Johne's shedders as well as clinical cases of Johne's disease. By focussing effort on identifying high-risk shedders that are spreading infection to young stock and becoming less productive, diagnostic testing can be a highly effective tool to manage JD risks in the herd and reduce the economic and welfare impact of JD.

[1] MAP – ***Mycobacterium avium paratuberculosis*** (or Johne's bacteria in this guide through common usage)
[2] PCR – Polymerase Chain Reaction
[3] ELISA – Enzyme-Linked Immunosorbent Assay

Johne's Disease tests available for cattle

While a range of tests is available both to detect Johne's bacteria or the immune response to infection, only PCR and ELISA tests are recommended for the routine detection of JD in dairy herds (Table 1 and Appendix 1). Other tests have either been largely superseded because of poor sensitivity (e.g. CFT, AGID and ZN smears) or are used primarily for research (e.g. faecal culture, IFN, histology).

Table 1. Tests for Routine Detection of JD in Dairy Herds

Test method	Sample	Purpose	Pros	Cons
ELISA	• Blood (serum or EDTA plasma)	• Confirm clinical JD. • JD control Screen dry stock e.g. carry-over cows, bulls, purchased cows.	• Relatively inexpensive • Good performance in advanced JD. • Good specificity • Cheaper than PCR	• Sampling cost as a screening test • May miss 10%+ of heavy shedders and clinical JD
ELISA	• Herd-test milk	• JD control Screen dairy herd to reduce calf exposure and clinical disease.	• Inexpensive and hassle-free test to find advanced JD cows to cull • Pooling reduces costs	• May miss up to 10-20% of cows that are heavy shedders or pre-clinical • Limited to lactating cows
PCR	• Faeces • Milk • Post mortem • Slurry	• Confirm clinical JD. • Identify presence of JD shedding in herd.	• Excellent test to confirm clinical diagnosis of JD.	• Relatively expensive • No indication of level of shedding in subclinical cattle
qPCR [quantitative PCR]	• Faeces • Milk • Post mortem • Slurry	• Confirm clinical JD. • JD control ID and rank Johne's shedders to prioritise culling. • Rank JD shedding in ELISA positives.	• Highly sensitive (if bacteria are present in the sample) and specific • 'Here-and-now' estimate of shedding to prioritise culling. • Can pool to reduce cost • Screen in-calf heifers	• More expensive than ELISA • May not detect early stage JD (if shedding is intermittent)

Test selection and other considerations

1. Confirming suspected cases of clinical Johne's disease

Suspected cases of clinical JD should be identified and culled as quickly as possible to avoid unnecessary expense and suffering. By monitoring changes in production closely (e.g. lactation value or LV), farmers are able to identify suspect cows before clinical signs develop. Confirmation testing before culling ensures accurate diagnosis and helps inform management decisions.

Ideally the test chosen should be highly sensitive and able to identify all clinical JD correctly. Both PCR and ELISA perform well in the later stages of the disease and are well suited to the task, although a small proportion of cows will remain ELISA negative even during the clinical phase.

2. Herd screening to reduce the impact of JD

Herd screening can be used to reduce the impact of JD in the herd by:

- eliminating shedders before calving to protect the next generation,
- culling cows before they develop clinical JD, and
- removing inefficient cows with failing production.

When selecting a test and testing regime for herds (Table 2), it is important to remember that no test will identify all Johne's cows; invariably some shedders will remain undetected and new cases will emerge. It is not possible to eliminate all risk and eradicate Johne's disease from a herd. The primary focus should be to reduce the JD risks whilst ensuring that the programme is sustainable and can be maintained on an annual basis i.e. testing regimes need to be practical and affordable.

Keep JD screening simple. Depending on the severity of JD in the herd and owner preferences, additional testing may be indicated.

Table 2. Herd screening options

Herd screeing options	
Primary Herd-Test Milk Screen	**Screen the whole herd using herd-test milk ELISA in late lactation.** Screening should be carried out before culling decisions need to be made and any cows have been dried off. This will identify most heavy shedders (>80%) in the herd as well as some low shedding cows. To minimise the spread of Johne's all test-positive cows should be culled if possible, including low shedders which may progress to heavy shedding or clinical JD by calving or during lactation.
Optional PCR Confirmation	**Test milk ELISA-positive cows to identify heavy shedders by qPCR.** High prevalence herds with limited scope to cull may find faecal qPCR a useful tool to prioritise culling decisions. qPCR will help rank cows by shedding level and identify heavy shedders for immediate removal. If low shedders are to be kept in the herd they should be calved separately and monitored closely for any signs of clinical JD. Cull their offspring and do not feed their colostrum to replacement calves.
Optional PCR Pooling	**ELISA-negative cows may be re-screened by pooled qPCR.** If a more intensive test-and-cull policy is required, pooled qPCR can be used to identify heavy shedders that are ELISA-negative.
Optional Heifer Screening	**In high Johne's challenge herds heifers should be screened before calving** Clinical JD amongst 1st lactation heifers suggests very high JD challenge in the herd and exposure of the next crop of calves is likely to be higher again. To help break this cycle of transmission, consider screening of in-calf heifers before calving by serum ELISA or faecal qPCR. Once JD incidence is reduced in the herd, this step may become unnecessary.

3. Herd screening to reduce the impact of JD

It is prudent to screen cattle for JD before purchase, particularly adult stock. Both qPCR and ELISA tests may be used.

Table 3. Testing to keep Johne's disease out of a Herd

Testing other stock	
Whole Herd	A pre-purchase whole-herd ELISA screen is the most efficient way to assess the Johne's status of a herd and allows test-positive cows to be excluded.
Heifers	In most cases, testing will not be able to identify infected young stock. Beware of a false sense of security if heifers test negative. Test-positive heifers in a herd suggest that exposure levels may be very high, which is an important warning sign. Good herd history or milking herd JD screen is likely to be more informative.
Carry-overs	Carry-over cows are high-risk animals. They should be tested before carrying over and again before calving. It is imperative that carry-over cows are not run with the replacement heifers at the run-off.
Bulls	All bulls should be tested for BVD and EBL. A Johne's ELISA on the same blood sample will safeguard against unnecessary JD risk.

Testing in Johne's Disease control programmes for dairy herds

Keep control programmes as simple yet effective as possible for ease of implementation and to ensure ongoing compliance in subsequent years. Whole herd screening of the adult cows is an essential part of a robust JD control programme. Seasonal calving patterns in New Zealand dairy herds offer an unique opportunity to screen the entire herd by herd-test milk ELISA at the same time i.e. before end-of-season culling and the next calving season.

Herd screening can be carried out at any stage throughout lactation. However while farmers may seek urgent solutions right after calving when most JD losses occur, screening in early lactation is of limited value unless the farmer is prepared to cull before peak lactation and repeat the screening before the following calving season. Ideally herd screening should be carried out as close to calving as possible to protect the next generation of heifers. Practical considerations however mean that testing needs to be carried out:

- before major culling decisions are made, and
- while the entire herd is still in milk (if the milk ELISA is to be used).

Split-calving herds may need to test earlier e.g. December. Herds in drought-prone areas herds may need to be tested by February, while others (especially SI herds) may delay testing until April.

PCR or serum ELISA screening can be useful if herds have already dried cows off and to test heifers or carry-overs if needed.

Table 4. Interpreting ELISA test results

ELISA Test Result	Interpretation
High-Positive	A high-positive result indicates a cow has JD and should be culled. Further confirmation testing tends to lower overall test sensitivity. Some high-positive cows may be shedding a smaller number of JD bacteria at the time of testing, but in time, especially after calving stresses, they may become heavy shedders in the next season.
Positive cows	A weaker positive result means a Johne's infection may be less advanced. Ideally all positive cows should be culled before calving if numbers allow it. Milk ELISA: Retesting by faecal PCR or serum ELISA will exclude any false positives due to contamination during herd-testing.
Suspect cows	Suspect cows should be retested to assess their JD status better.
'No Antibody Detected' or Negative	Because the ELISA will not detect early JD infections, negative test results are also reported as 'No Ab detected'. Cows should be retested annually to detect changes to their JD status because they may be shedding MAP or advancing towards clinical JD by the following season. A small proportion of cows with advanced JD may evade detection by ELISA but farmers should expect clinical JD to be reduced by 80 to 100% after rigorous pre-season test-and-culling.

APPENDIX 1

1. Herd-Test Milk ELISA Screening

Herd testing provides a convenient and inexpensive opportunity to screen the whole herd so that high-risk JD cows can be culled before calving. This will help to limit exposure of replacement heifer calves and also to minimise clinical JD losses in the milking herd during the next season.

Milk samples are tested by ELISA. An initial pooling step with reduced test cut-off (to ensure test sensitivity remains unaffected) minimises the cost of screening the whole herd. Each milk sample from a positive pool is retested so that every cow receives an individual test result.

Performance of the milk ELISA is similar to serum ELISA testing although carryover contamination during herd-testing can cause some false suspect to weak positive results. By grouping ELISA results into high-positive vs weaker positives, the impact of contamination on test specificity can be managed while also giving a better assessment of the likely shedding by the cow.

Johne's bacteria shedding is heaviest amongst high-positive ELISA cows

Between 83% (by faecal culture) and 91% (by faecal PCR) of high-positive milk ELISA cows in a JDRC[4] trial had a positive faecal sample compared with 47% and 58% of cows with weaker positive milk results. PCR results also show that a much higher proportion of high-positive ELISA cows (55%) were shedding moderate to heavy numbers of MAP, see below.

Performance of the Johne's ELISA tests compared with faecal culture or PCR

	n	Serum ELISA	Faecal Culture	Faecal PCR (quantitative)		
		Positive	Positive	mod-heavy	low shedding	not detected
Herd-test Milk Johne's ELISA PPV*:						
'High positive cows' only	233	98%	83%	55%	36%	9%
Remaining 'positive cows'	47	64%	47%	11%	47%	42%
All milk ELISA 'positive cows'	280	92%	77%	48%	37%	15%
Serum Johne's ELISA PPV*						
'High positive cows' only			86%	56%	36%	8%
Remaining 'positive cows'			60%	15%	55%	30%

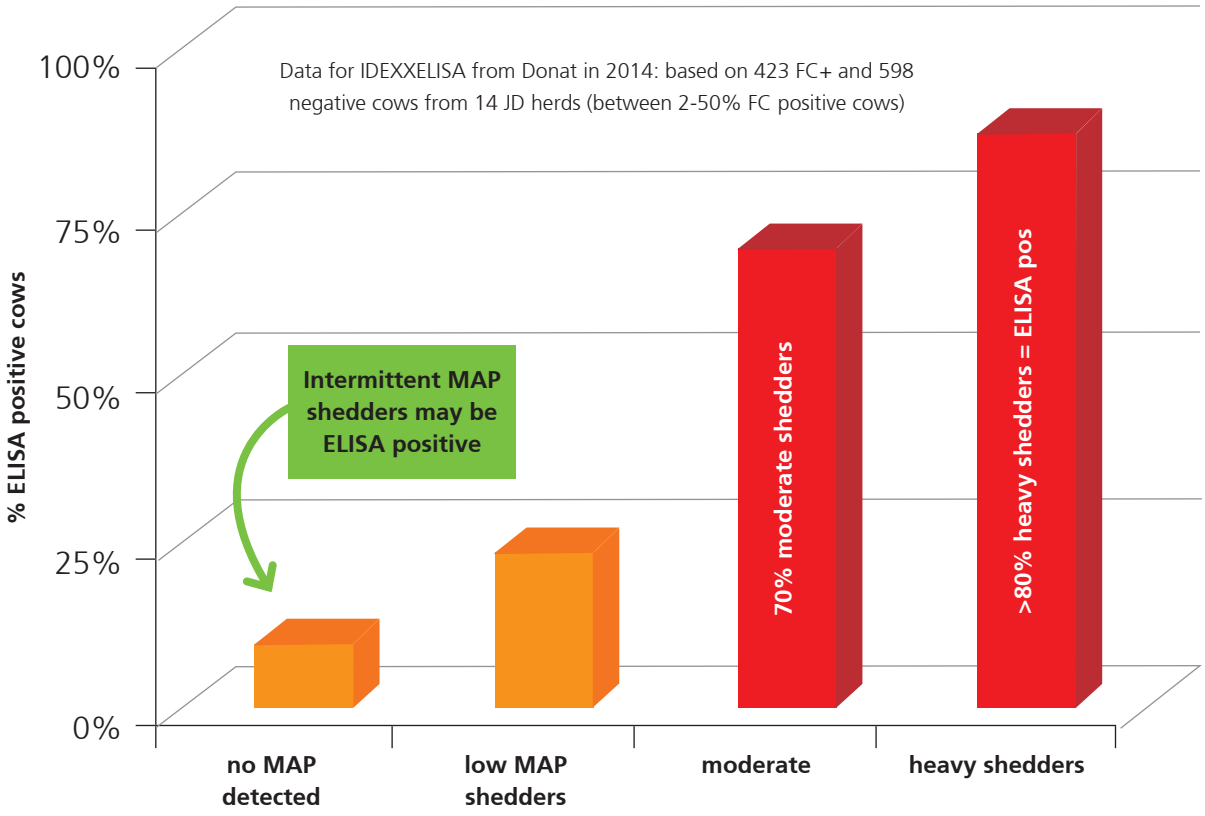
*** PPV = Positive Predictive Values: % Johne's ELISA-positive cows that tested positive by confirmation tests**
Data from a JDRC trial with 280 herd-test milk ELISA positive cows in 26 New Zealand dairy herds.

[4] JDRC

2. ELISA Blood Testing

The ELISA kits currently used by laboratories in New Zealand all have very high test specificity (>99%) for serum or plasma (EDTA) samples. However, test sensitivity of both the milk and serum ELISA is highly dependent on the population of cattle sampled: Amongst young stock and during early stages of the disease, or in herds with low infection challenge, test sensitivity may be well below 20%. So testing clinically 'healthy' cattle with the aim to identify every JD infected individual can be very inefficient. On the other hand, ELISA tests are good at detecting advanced stages of JD with test sensitivity reaching well over 80% in heavily shedding and clinical JD cattle (Figure 1). By focussing on the high-risk animals with advanced JD, test sensitivity is good and risk based ELISA testing becomes a highly effective tool to help combat JD in the herd.

Figure 1.



3. Quantitative PCR Testing

PCR results reported simply as positive or negative are only useful to confirm diagnosis of clinical Johne's disease. Quantitative PCR on the other hand means the number of Johne's bacteria in the sample can be quantified which gives the best measure of a cow's shedding level at that point in time. Heavy shedders excrete massive amounts of Johne's bacteria; so that contamination from a single heavy shedder may be equivalent to thousands of low shedding cows. Central to any JD control programme designed to reduce calf exposure, is identification and culling of Johne's shedders – especially heavy shedding cows. qPCR allows active shedders to be ranked in order of risk which helps to inform and expedite culling decisions; see Table 5 for PCR risk categories as used by Disease Research Laboratory (DRL).

Table 5. Risk categories for qPCR

qPCR Test Result Interpretation		
Not Detected	$<10^2$ gme*	Good
Background	$\geq10^2$ - $<10^3$ gme	Monitor cows (in herds with high JD shedding, pass-through contamination with false positive result is possible in some cows)
Low	$\geq10^3$ - $<10^4$ gme	Cull if possible; or tag to manage risks & monitor
Moderate	$\geq10^4$ - $<10^5$ gme	Cull if possible; or tag to manage risks & monitor
High	$\geq10^5$ - $<10^6$ gme	Cull ASAP or before calving
Very High or 'Super-shedders'	$\geq10^6$ gme	Cull ASAP

*gme – a relative measure of JD bacteria in a sample (genomes per mL PCR reaction volume, used by DRL)

Faecal samples are easily taken by lay personnel and a tablespoon of faeces is sufficient for the assay. PCR can be used at any time through the year and may also be used on young animals. PCR tests are more expensive than ELISA tests, but the nature of the assay and huge range of faecal shedding also lend itself to pooling samples to reduce the cost of sampling. This test is likely to give a more accurate assessment of shedding amongst young stock than the ELISA test and may be used in severely affected herds to screen heifers prior to first calving. However test sensitivity (the ability to detect any JD infected individual) is reduced in animals with early stage JD when shedding may be intermittent or not occurring.

The qPCR may also be used as an add-on following ELISA screening, particularly in herds with high ELISA test-prevalence where culling of all positives is not feasible. Follow up testing of ELISA positive cows with qPCR will enable the farmer to prioritise culling of the worst affected (heavy shedding) cows while applying other risk management options for low shedders i.e. separate calving etc.

APPENDIX 2

How long do JD test-positive cows survive?

A case study

During pooled ELISA test validation for a JDRC trial in March 2008, more than 800 milk samples from 5 to 10 year-old cows in a herd were tested without the farm manager knowing the results. Final cull and fate records for the cows were extracted from the MINDA database and analysed six years later (Table 4). The results showed that:

- A third of the high-positive cows died during calving with signs of clinical JD observed by the farmer.
- Within 13 months, half of the high-positive cows were dead compared with 27 months for ELISA negatives.
- Overall, test positive cows were far more likely to die than be sent to the works compared with the rest of the herd.

Note: During the following spring calving another two (of > 800 test negative) cows died with signs of Johne's disease (MINDA recording). It is important to remember that testing can drastically reduce Johne's risks but will not detect all JD cows. Therefore JD control requires an integrated risk-management programme to protect replacement stock.

Survival time (in months) from test date to final date (death or sold / sent to slaughter):

	n (cows)	Median survival (months)	Odds of fate = died vs sold	Comment
High POS	15	13	1.1	5 died during calving Aug-Oct (JD)
Positive	11	20	0.7	
Negative	843	27	0.2	2 died during calving Aug-Oct (JD)

Reference Selection

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O'Brien R, Hughes a, Liggett S and Griffin F (2013) Composite testing for ante-mortem diagnosis of Johne's disease in farmed New Zealand deer: correlations between bacteriological culture, histopathology, serological reactivity and faecal shedding as determined by quantitative PCR. BMC Veterinary Research 9 (72), 1746-6148.

Test providers

DRL (Disease Research Laboratory)	Phone 03 489 4832 Block C, Invermay Agricultural Centre,176 Puddle Alley, Mosgiel 9053 www.drl.otago.ac.nz and drl@otago.ac.nz
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LIC Diagnostics	Phone 0800 GENEMARK (0800 436 362) 140 Riverlea Road, Riverlea, Hamilton 3216 www.lic.co.nz and genemark@lic.co.nz
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